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GENE EXPRESSION SIGNATURES OF cAMP/PKA-PROMOTED, MITOCHONDRIAL-DEPENDENT APOPTOSIS: COMPARATIVE ANALYSIS OF WILD-TYPE AND cAMP-DEATHLESS S49 LYMPHOMA CELLS

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The second messenger cAMP acts via protein kinase A (PKA) to induce apoptosis by mechanisms that are poorly understood. Here, we assessed a role for mitochondria and analyzed gene expression in cAMP/PKA-promoted apoptosis by comparing wild-type (WT) S49 lymphoma cells and the S49 variant, D- (cAMP-deathless), which lacks cAMP-promoted apoptosis but has wild-type levels of PKA activity and cAMP-promoted G_1 growth arrest. Treatment of WT, but not D-, S49 cells with 8-CPT-cAMP for 24 h induced loss of mitochondrial membrane potential, mitochondrial release of cytochrome c and Smac and increase in caspase-3 activity. Gene expression analysis (using Affymetrix 430 2.0 Arrays) revealed that WT and D- cells incubated with 8-CPT-cAMP have similar, but non-identical, extents of cAMP-regulated gene expression at 2h (~800 transcripts) and 6h (~1000 transcripts) (|Fold|>2, P<0.06); by contrast, at 24h ~2500 and ~1100 transcripts were changed in WT and D- cells, respectively. Using an approach that combined regression analysis, clustering and functional annotation to identify transcripts that showed differential expression between WT and D- cells, we found differences in cAMP-mediated regulation of mRNAs involved in transcriptional repression, apoptosis, the cell cycle, RNA splicing, Golgi and lysosomes. The 2 cell lines differed in CREB phosphorylation and expression of the transcriptional inhibitor Icer and in cAMP-regulated expression of genes in the Inhibitor of apoptosis (IAP) and Bcl families. The findings indicate that cAMP/PKA-promoted apoptosis of lymphoid cells occurs via mitochondrial-mediated events and imply that such apoptosis involves gene networks in multiple biochemical pathways.

The ability of the second messenger cAMP to alter the balance between cell growth and apoptosis is cell type-dependent: cAMP stimulates growth and inhibits apoptosis in some cell types, such as neuronal cells(1) but it promotes growth arrest and apoptosis in other types of cells, such as poorly differentiated lymphoid cells (2). In addition, cAMP analogs can enhance the pro-apoptotic effects of glucocorticoids, for example, of glucocorticoid-resistant multiple myeloma and leukemia cells (2-5). However, the mechanisms that mediate cAMP-induced apoptosis are poorly understood.

Cyclic AMP-promoted apoptosis of lymphoid, in particular T-cell-derived lymphoma, cells depends on the principal effector of cAMP action, protein kinase A (PKA) (2-4,6). Although both PKA and the exchange protein directly activated by cyclic AMP (Epac), another mediator of cAMP action, are found in lymphoid cells, Epac does not appear to be involved in cAMP-mediated control of the cell cycle (7) or apoptosis in these cells (2,8). Overexpression of certain anti-apoptotic proteins, such as Bcl-2, can protect lymphoma cells from cAMP-mediated apoptosis; such protection appears to be distinct from effects of cAMP on cell cycle arrest (9). Thus, cAMP-